Comparison of the microbial communities in anaerobic digesters treating high alkalinity synthetic wastewater at atmospheric and high-pressure (11 bar)

Zhao, Jing; Li, Yu; Marandola, Clara; Krooneman, Janneke; Euverink, Gert Jan Willem

Published in:
Bioresource Technology

DOI:
10.1016/j.biortech.2020.124101

Publication date:
2020

Bioresource Technology 318 (2020) 124101

Contents lists available at ScienceDirect

Bioresource Technology

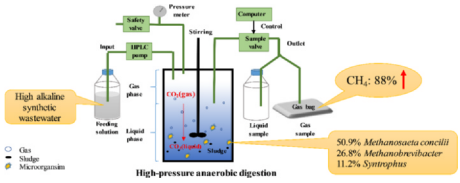
journal homepage: www.elsevier.com/locate/biortech

Comparison of the microbial communities in anaerobic digesters treating high alkalinity synthetic wastewater at atmospheric and high-pressure (11 bar)

Jing Zhao[a], Yu Li[a], Clara Marandola[a], Janneke Krooneman[a,b], Gert Jan Willem Euverink[a,b,*]

[a] Faculty of Science and Engineering, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
[b] Carbohydrate Competence Center, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
High-pressure anaerobic digestion
Alkalinity
Volatile fatty acids
Syntrophic bacteria
Methanosaeta concilii

ABSTRACT

High-pressure anaerobic digestion is an appealing concept since it can upgrade biogas directly within the reactor. However, the decline of pH caused by the dissolution of CO_2 is the main barrier that prevents a good operating high-pressure anaerobic digestion process. Therefore, in this study, a high-pressure anaerobic digestion was studied to treat high alkalinity synthetic wastewater, which could not be treated in a normal-pressure anaerobic digester. In the high-pressure reactor, the pH value was 7.5 ~ 7.8, and the CH_4 content reached 88% at 11 bar. Unlike its normal-pressure counterpart (2285 mg/L acetic acid), the high-pressure reactor ran steadily (without volatile fatty acids inhibition). Furthermore, the microbial community changed in the high-pressure reactor. Specifically, key microbial guilds (*Syntrophus* (11.2%), *Methanosaeta concilii* (50.9%), and *Methanobrevibacter* (26.8%)) were dominant in the high-pressure reactor at 11 bar, indicating their fundamental roles under high-pressure treating high alkalinity synthetic wastewater.

1. Introduction

Biogas produced by conventional anaerobic digestion (AD) is primarily composed of methane (CH_4, 50 ~ 70%) and carbon dioxide (CO_2, 30 ~ 50%), and the ratio mainly depends on the substrate and pH of the fermentation process in the AD reactors (Wahid et al., 2019). The application possibilities of biogas are limited due to its low calorific value, which is caused by the low ratio of CH_4/CO_2 (Lemmer et al., 2017; Lindeboom et al., 2011). Before injecting biogas into the natural gas grid or used in other high-grade applications (CH_4 > 90%, CO_2 < 8%), upgrading technologies are necessary (Omar et al., 2018; Wahid et al., 2019). External upgrading technologies, such as cryogenic, chemical absorption, membrane separation, pressure swing adsorption, water scrubbing, are applicable only for biogas flows higher than 100 m^3/h (Angelidaki et al., 2018; Li et al., 2017; Lindeboom et al., 2011). For small-scale digesters mounted in the so-called Decentralized Sanitation and Reuse (DeSaR) system, the upgrading equipment is neither available nor cost-effective (Li et al., 2017; Lindeboom et al., 2011). Thus, there is a demand for the development of new technologies that improve the biogas quality at a small scale.

* Corresponding author at: Faculty of Science and Engineering, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands.
E-mail address: g.j.w.euverink@rug.nl (G.J.W. Euverink).

https://doi.org/10.1016/j.biortech.2020.124101
Received 11 July 2020; Received in revised form 1 September 2020; Accepted 3 September 2020
Available online 08 September 2020

Table 1
Literature review on high-pressure anaerobic digestion.

Reactors	Conditions	Substrates	Process performance	Archaea (domain)	Reference
Single stage	30 °C, batch	$NaCH_3COO{\cdot}3H_2O$	CH_4 content was 90 ~ 95% at a pressure of 3–90 bar. The SMA decreased by 30% compared to atmospheric conditions.	None	Lindeboom et al., 2011
Single stage	30 °C, batch	VFAs	Pressure:1 ~ 20 bar; CH_4 > 94% Substrate and cation inhibition reduce conversion rates.	None	Lindeboom et al., 2013b
Single stage	30 °C, batch	Sodium acetate	Pressure:1 ~ 20 bar; CH_4 > 95% (pH ~ 7); CH_4 > 80% (pH 5 ~ 6) pCO_2 below ideal theoretical equilibrium.	None	Lindeboom et al., 2012
Single stage	30 °C, batch	Glucose	Pressure:1 ~ 10 bar; CH_4 75 ~ 88% Add silicate can buffer glucose acidification and sequestrate CO_2.	None	Lindeboom et al., 2013a
Single stage	30 °C, batch	Sodium acetate, Glucose, propionate	Pressure:1 ~ 20 bar, pH 6.5 ~ 7 pCO_2 could inhibit propionate degradation (5 bar).	*M. concilii;* *M. formicicum* (DGGE)	Lindeboom et al., 2016
Single stage, CSTR	37 °C	Activated sludge	Pressure:1 ~ 6 bar, CH_4 85%, phosphate solubility increased, COD removal decreased.	*Methanocellaceae*	Latif et al., 2018
Two stage, Methane filter reactor	37 °C, batch	A mixture of grass and maize silage hydrolysate.	10, 20, 30 bar, and 1, 50, 100 bar The initial pressures do not have significant influence on pressure increase, degradation of organics and SMY.	None	Lemmer et al., 2017; Merkle et al., 2017a
Two stage, Methane filter reactor	37 °C, continuous	Maize silage	Pressure: 9 ~ 50 bar; flow rate: 0, 20, 40 L/day Water scrubbing can help to increase pH from 6.5 to 6.7, and CH_4 increase from 75% to 87%.	None	Lemmer et al., 2015b; Merkle et al., 2017b
Two stage, Methane filter reactor	37 °C	Leachate from maize silage/grass and maize silage.	Pressure: 1, 3, 6, 9 bar pH from 7.2 to 6.5, CH_4 from 66 to 75%, and reduced SMY. Higher NH_4 lead to higher pH and CH_4 content, but lower SMY.	None	Chen et al., 2014a; Lemmer et al., 2015a
Two stage, Methane filter reactor	37 °C	leachate from maize silage OLR: 5 ~ 17.5 kg m^3d^{-1}	Pressure: 1.5, 9 bar; CH_4: 66.2, 74.5%; pH 6.5 at 9 bar Best performance was 9 bar and 12.5 kg m^3d^{-1}. Higher OLR becomes unstable.	None	Chen et al., 2014b
Two stage, Biofilm reactor	37 °C, continuous	Food waste	Pressure: 3 ~ 17 bar, pH decreased from 7.22 to 6.98, COD removal decreased 93 to 80%, CH_4 increased 80 to 91%, SMY decreased.	*Methanosaeta,* *Methanospirillum*	Li et al., 2017

Notes: SMY: specific methane yields, SMA: specific methane activity, OLR: organic loading rate.

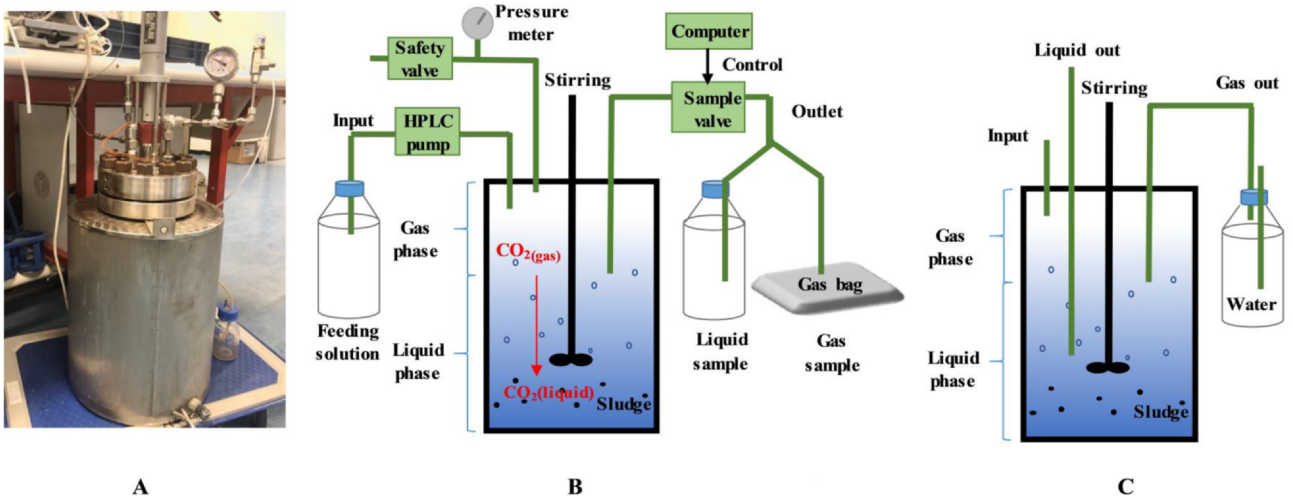


Fig. 1. (A) Photograph of the HPAD reactor. (B) Schematic view of the HPAD reactor. (C) Schematic view of the NPAD reactor. Notes: For the HPAD reactor, an HPLC pump was used for feeding, a sample valve used for liquid and gas samples collection, was controlled by a computer (When the tube was inside the liquid phase, liquid samples were taken; when the tube was above the liquid, gas samples were taken, which is a technical set up mentioned previously.) For the NPAD reactor, the water displacement method was used for the determination of the biogas production. One tube was used for taking liquid samples and the other one was used for feeding.

Lindeboom et al. (2011) proposed a novel AD concept: high-pressure anaerobic digestion (HPAD), which integrates biogas generation, upgrading and pressurisation in one step and hence it can condiserably reduce capital expenditures (Budzianowski and Postawa, 2017). Based on Henry's law, CO_2 dissolves better than CH_4 in the liquid phase when the pressure increases. Hence, the CH_4 content in the gas phase increases under pressure. The Henry's constant for CH_4, CO_2, H_2S, and NH_3 are 0.0016, 0.0318, 0.115 and 62 mol/L/bar (0 °C and 1 atm), respectively (Lindeboom et al., 2011). In the last decades several studies have been published investigating single stage HPAD reactors or two-stage presssurized reactors to upgrade biogas (Table 1). Specifically, the CH_4 content could reach more than 90% at pressures above 3 bar, but the pH drops at the same time, that could influence methanogens activity (Lindeboom et al., 2011, 2012). Moreover, the pressure itself may impose an influence on methanogenic activity as well. It was previously shown that some methanogens that were present in atmospheric AD could maintain their activity at pressures up to 90 bar (Lindeboom et al., 2011). These archaeal OTUs were closely related to *Methanosaeta concilii, Methanosarcina acetivorans, Methanobacterium formicicum* and *Methanobacterium beijingense* (Lindeboom et al., 2016). In another study, *Methanosaeta* (47%) and *Methanospirillum* (32.4%) dominated at 17 bar in a two-stage biofilm reactor, and the pressure was negatively correlated with the microbial diversity (Li et al., 2017). In spite of these harsh conditions, these studies revealed that some methanogens managed to survive and served as the main methane contributor.

On the other hand, the decline of pH caused by the dissolution of CO_2 prevented stable operation of these high-pressure AD reactors (Table 1). Therefore, some alternative operations are suggested to alleviate the impact of CO_2 in HPAD. In previous studies, water scrubbing and silicate addition were used to remove CO_2 from the liquid phase, and could also further increase the CH_4 content (Lemmer et al., 2015b; Lindeboom et al., 2013a), but these approaches require extra investment. Intriguingly, in some studies using atmospheric AD treating a waste stream containing high alkalinity (high pH) some problems were encountered. Particularly, alkaline pretreament is commonly used to enhance biogas production from lignocellulosic biomass, but the hydrolysate with high alkaline (7.5% NaOH) content inhibited the subsequent methanogenesis stage in ambient AD (Zhu et al., 2010). These problems were also experienced when brewery wastewater/wastes (two thirds of the wastewater from the brewery is alkaline, pH 9 ~ 12) (Rao et al., 2007), or excess sludge (Li et al., 2018) was treated in ambient AD. It suggests that HPAD might be an alternative to treat these kind of alkaline waste streams because a suitable pH for operation can be maintained through the dissolved CO_2. Under such circumstances, the microbial community within the HPAD remains poorly documented and requires more investigation.

Thus, in this study, a mesophilic HPAD single-stage bioreactor was operated at moderate high pressure, not exceeding 11 bar, and fed with high alkaline synthetic wastewater containing acetic acid, sodium acetate or glucose. The aims were to (1) evaluate the process stability to produce high calorific biogas in an HPAD system, (2) illustrate the microbial community using metagenomics genetic tools (16S-rDNA gene sequencing) and (3) analysis of the VFAs content in the reactors in relation to the total alkalinity. Hereto the results were compared with the methane yield and microbial diversity in a normal-pressure anaerobic digester (NPAD) reactor.

2. Materials and methods

2.1. Reactor set-up

For the high-pressure experiment, an HPAD reactor (Suurmond, Switzerland) with a total volume of 2.0 L was used, and the liquid phase was manually controlled at 1.2 ~ 1.5 L. The reactor was designed for operation up to 300 bar in a temperature range of 0 °C ~ 350 °C. This HPAD reactor was equipped with a pressure meter, an HPLC pump (Thermo, SpectraSystem P4000, Netherlands) for substrate feeding, a sample valve controlled by a computer, and a mechanical safety valve (Fig. 1A, B). The liquid and gas samples were taken using the same sample valve. When the sample tube was below the liquid phase, liquid samples were taken; when the sample tube was above the liquid phase, gas samples were collected using a gas bag.

For normal-pressure (atmospheric pressure) experiments, a glass NPAD reactor with a total volume of 2.0 L was used, and the volume of the liquid phase was the same as the HPAD reactor (1.2 ~ 1.5 L) (Fig. 1C). The temperature of the reactors was maintained at 37 ± 1 °C with a stirring speed of 100 rpm.

2.2. Experimental start-up and operation

The reactors were inoculated with anaerobic sludge from a full-scale

Table 2
Overview of the HPAD reactor and the NPAD reactor.

	Phase	Duration (days)	Liquid volume (L)	Gas volume (L)	Liquid/ gas ratio	Substrate	Total COD (g)	Estimated total CH_4 production [a] (L)	Pressure initial (bar)	Pressure final (bar)	CH_4% [b]	Acetic acid (mg/L)
HPAD	1	0 ~ 11	1.50	0.50	3.00	None			0	1.9	ND	–
	2	11 ~ 27	1.64	0.36	4.56	CH_3COOH	11.2	3.14	1.9	10.1	ND	–
	3	27 ~ 32	1.55	0.45	3.44	CH_3COOH	4.0	1.12	8.0	10.6	ND	–
	4	32 ~ 37	1.49	0.51	2.92	CH_3COONa	2.9	0.81	8.0	11.0	ND	–
	5	37 ~ 44	1.39	0.61	2.28	CH_3COONa	4.1	1.15	7.9	10.9	ND	–
	6	44 ~ 52	1.29	0.71	1.82	CH_3COONa	4.7	1.86	8.0	11.0	87.4	14
	7	52 ~ 59	1.16	0.84	1.38	$C_6H_{12}O_6$	5.6	2.16	8.0	10.9	88.5	–
	Phase	Duration (days)	Liquid volume (L)	Gas volume (L)	Liquid/ gas ratio	Substrate	Total COD (g)	Total CH_4 production [c] (L)	CH_4%	Acetic acid (mg/L)	Propionic acid (mg/L)	Butyric acid (mg/L)
NPAD	1	0 ~ 11	1.50	0.50	3.00	No			48.5	–	–	–
	2	11 ~ 27	1.64	0.36	4.56	CH_3COOH	11.2	2.17	64.0	–	–	–
	3	27 ~ 32	1.55	0.45	3.44	CH_3COOH	4.0	0.50	64.3	–	–	–
	4	32 ~ 37	1.49	0.51	2.92	CH_3COONa	2.9	0.28	55.5	142	–	–
	5	37 ~ 44	1.39	0.61	2.28	CH_3COONa	4.1	0.20	56.5	2285	–	–
	6	44 ~ 52	1.29	0.71	1.82	$C_6H_{12}O_6$	6.4	0.39	42.2	3212	178	18
	7	52 ~ 59	1.16	0.84	1.38	$C_6H_{12}O_6$	5.6	0.45	35.6	7455	451	52

"–" means below the detection limit of the HPLC method. Propionic and butyric acid in the HPAD reactor were below the detection limit of the HPLC method.

[a] In the HPAD reactor, the estimated total CH_4 production in phase 2 to 5 was calculated using the total COD fed into the reactor during that phase (1 g COD yields 0.35 L CH_4; 80% COD is converted into biogas, the other 20% is used for cell growth (Filer et al. 2019)). The estimated total CH_4 production in phase 6 and 7 was calculated by CH_4 content (CH_4 production = pressure increased × headspace × CH_4%).

[b] The methane content of the biogas in the HPAD reactor was measured at the end of phase 6 and 7 due to the technology set up.

[c] In the NPAD reactor, the actual total CH_4 production was calculated using the CH_4 content in the biogas. "ND" means not determined, because of technology set up. The HPAD reactor has one combined outlet for liquid and gas. When the tube was inside the liquid phase, liquid samples were taken; when the tube was above the liquid, gas samples were taken.

anaerobic digester treating aerobic sludge from a wastewater treatment plant (Garmerwolde, The Netherlands). The composition of synthetic wastewater was as follows: NH_4Cl 2.0 g/L, $K_2HPO_4{\cdot}3H_2O$ 3.6 g/L, KH_2PO_4 2.8 g/L, $NaHCO_3$ 5.0 g/L, 1.0 mL/L of trace element solution, 1.0 mL/L of Wolfe's vitamin solution, and 1.0 mL/L cysteine-sulfide reducing agent dissolved in deionized water (Ronald, 2004). The components, salts and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA), Fisher scientific (Acros organics, USA) and Merck (KGaA, Germany). The synthetic wastewater was used as substrate with the same concentration (75 g/L) of acetic acid, sodium acetate, or glucose as carbon source during different phases, respectively (Table 2).

The overall experiment was divided into seven phases (Table 2). Phase 1: cultivation phase, the sludge produced biogas and adapted to autogenerated pressure; phase 2–3: adaptation to autogenerated pressure conditions on acetic acid (pH of the synthetic wastewater was adjusted to 7 using 5 M NaOH and the total alkalinity was 18.6 g/L as $CaCO_3$); phase 4–6: pressure operation on sodium acetate/glucose (pH of the synthetic wastewater was adjusted to 7 using 37% HCl and the total alkalinity was 14.4 g/L as $CaCO_3$); phase 7: pressure operation on glucose.

Synthetic wastewater (10 mL) was daily added using an HPLC pump and a syringe for the HPAD and NPAD reactors, respectively. The effluent samples were taken at the end of each phase. For the HPAD reactor, the sample valve was controlled by a computer using a '10 s closed, 1 s open' procedure until the required sample volume was collected. From phase 2 on, when the pressure increased up to 10 ~ 11 bar, the samples were taken until the pressure decreased to 8 bar. The volume of the effluent samples was dependent on the pressure. For the NPAD reactor, the same sample volume was obtained using a syringe.

2.3. Gas production calculation

The volume of daily biogas production (DBP) of NPAD was measured by the water displacement method, and the volume of daily methane production (DMP) was calculated by DBP × CH_4%.

For the HPAD reactor, it is hard to precisely calculated DBP and DMP, due to the lack of daily biogas composition data. Therefore, the estimated total CH_4 production in phase 2–5 was calculated using the total COD fed into the reactor during the corresponding phase (1 g COD yields 0.35 L CH_4; 80% COD is converted into biogas, the rest 20% is used for cell growth (Filer et al., 2019)). The estimated total CH_4 production in phase 6–7 was calculated based on CH_4 content (CH_4 production = pressure increased × headspace × CH_4%).

2.4. Physical and chemical analyses

Total solids (TS) and volatile solids (VS) were determined by the standard methods (APHA, 2005). The pH was measured using a digital pH meter (H160, Hach, Germany). The total volatile fatty acids (VFAs) and total alkalinity (TA) were analyzed by titration with 0.1 N H_2SO_4 to the endpoints of pH 5.0 and 4.4 with an auto-titrator (AT1000, Hach, Germany). The VFAs were analyzed with high-performance liquid chromatography (Agilent Technologies 1200 series) equipped with a Bio-Rad Aminex HPX-87H 300 × 7.8 mm column at 60 °C using 5 mM H_2SO_4 as eluent (0.5 mL/min) and detected using a UV-detector at 210 nm. The biogas composition was analyzed by gas chromatography (C2V-200 Micro GC, Thermo Scientific) with a GCC200-U-BND cartridge and a thermal conductivity detector. The temperature of the column, injector, and the detector was 60 °C, 120 °C, and 120 °C, respectively. Helium was used as carrier gas.

2.5. High-throughput 16S-rDNA gene sequencing and analysis

The samples (2 mL) were thawed at room temperature and total DNA was extracted using the FastDNA® Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer's protocol. The extracted genomic DNA was used as the template in the PCR reactions. Part of the 16S-rDNA genes was amplified using the primers 27F (5′-AGR GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-GGG TTA CCT TGT TAC GAC TT-3′ for Bacteria, and multiplexed with Arch21F (5′-TTC CGG TTG ATC CYG CCG GA-3′) for Archaea. The amplified DNA was

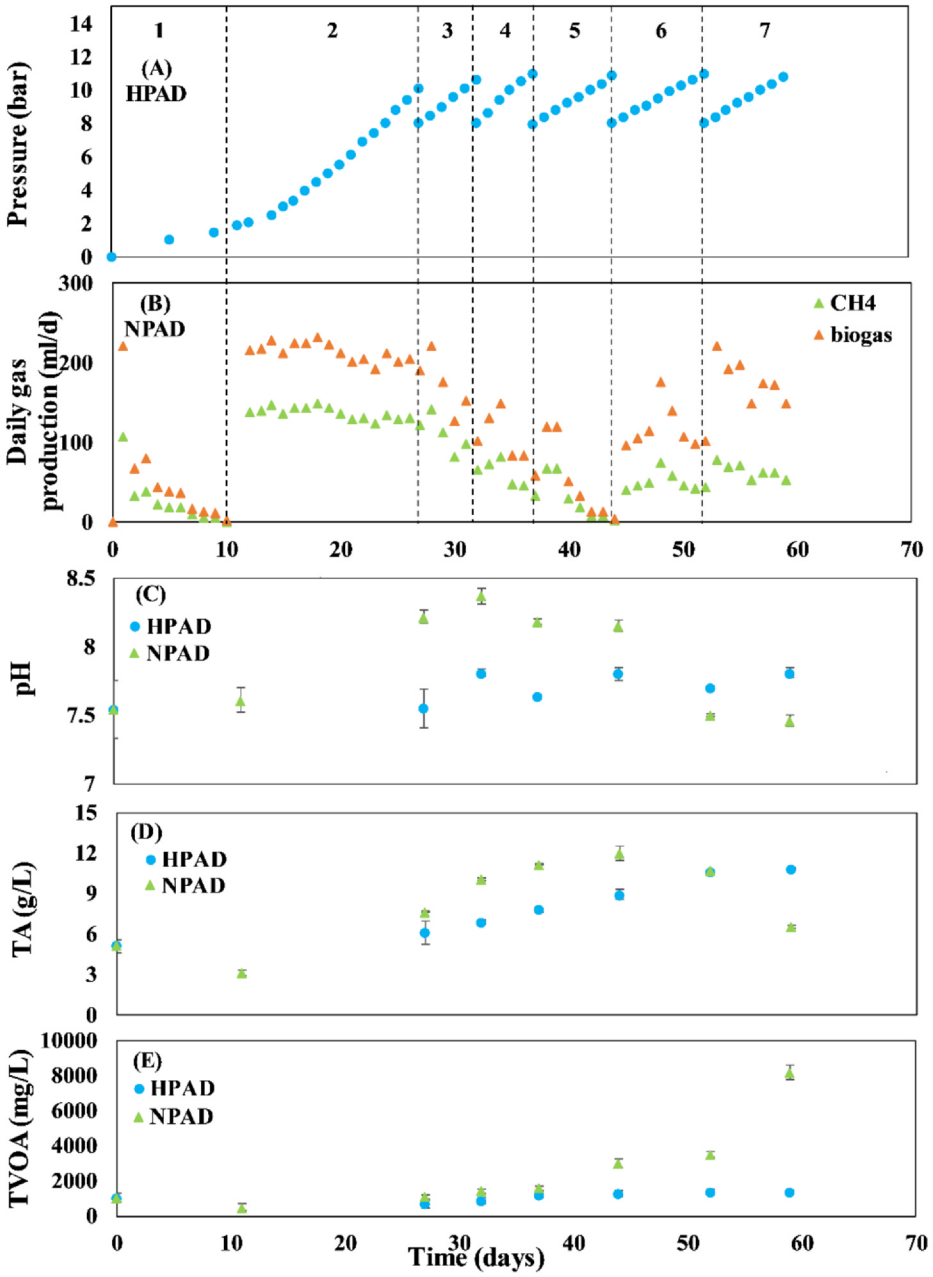


Fig. 2. (A) Pressure build-up in the high-pressure anaerobic digestion (HPAD) reactor. (B) Daily gas production of the normal-pressure anaerobic digestion (NPAD) reactor. (C) pH, (D) TA, and (E) TVOA in HPAD and NPAD reactors.

sequenced, using bTEFAP PacBio Sequel sequencing technology (MR DNA, Shallowater, Texas, USA). Diversity profiles were obtained by plotting the number of 16S-rDNA sequences of a species as a percentage of the total 16S-rDNA sequences in a sunburst graph.

3. Results and discussion

3.1. Autogeneration of biogas pressure

The profiles of the pressure in the HPAD reactor and DBP/DMP in the NPAD reactor are plotted in Fig. 2. In phase 1, the increase of pressure in the HPAD reactor and the production of biogas in the NPAD reactor came from the residual biomass in the inoculum sludge. When the pressure became stable in the HPAD reactor and DBP/DMP became zero in the NPAD reactor, the synthetic wastewater was added into both reactors.

In phase 2, acetic acid solution was added into the reactors and was directly utilized by the methanogens. During this phase, the HPAD and NPAD reactors both ran steadily. The pressure in the HPAD reactor increased from 1.9 to 10.1 bar. The DMP of the NPAD reactor reached around 135 mL/d and declined slightly afterwards. The total methane produced in phase 2 for the HPAD and NPAD reactor was around 3.14 L and 2.17 L, respectively (Table 2). At the end of phase 2, the pressure of the HPAD reactor dropped to 8 bar due to the extraction of liquid samples.

In phase 3, for the HPAD reactor, the pressure increased from 8.0 to 10.6 bar. While the DMP of the NPAD reactor decreased from 141 to 65 mL/day (Fig. 2B), which was due to the inhibition by a high TA (10 g/L). Therefore, in this context, the HPAD reactor was advantageous over the NPAD reactor when dealing with high alkaline acetic acid-containing synthetic wastewater.

From phase 4 to phase 6, the substrate was changed to a sodium

acetate solution for both reactors. The TA of the sodium acetate synthetic wastewater (14.4 g/L) was lower than that of the acetic acid synthetic wastewater (18.6 g/L). The adjustment of the substrate was necessary because a high pH value (8.2 ~ 8.4) and high TA occurred at the end of phase 2 and 3, especially in the NPAD reactor (Fig. 2). The pressure profiles from phase 4 to phase 6 followed a similar pattern in the HPAD reactor, except for the time needed to reach a pressure of 11 bar. The gas production rate was 0.20, 0.19 and 0.21 $L/d{\cdot}L^{-1}$, respectively for phase 4, 5 and 6. More time was needed for the pressure to reach 11 bar because of the increase of the headspace and the decrease of the liquid volume (Table 2). As a consequence, the pressure inside the reactor increased slower (more biogas is required to fill the remaining headspace). For the NPAD reactor, although the substrate was changed to sodium acetate solution, the DMP was not restored. Furthermore, it completely ceased at the end of phase 5 (Fig. 2B), indicating that the microbial community, especially methanogens, were inhibited. Presumably, the inhibition of the methanogens was caused by the high TA, which will be discussed in the next Section 3.2. From phase 6 on, the substrate in the NPAD reactor was changed to glucose, a substrate that does not require pH adjustment before use. The DMP of the NPAD reactor recovered to around 49 mL/d. But the cumulative methane production of the NPAD reactor (0.39 L) in phase 6 was still much lower than that of the HPAD reactor (1.86 L).

In order to compare the HPAD reactor with the NPAD reactor, the substrate of the HPAD reactor was also changed to glucose in phase 7. The HPAD reactor continued to produce biogas, leading to the increase of the pressure. In contrast, the DMP in the NPAD reactor decreased again. Perhaps, the inhibition of the methanogens in the NPAD reactor was too harsh for them to recover.

Apart from the methane yield, the methane content is another important parameter. Due to the technology set up, the biogas samples from the HPAD reactor were taken only at the end of phase 6 and 7. In a separate preliminary experiment with glucose as the substrate, the biogas in the headspace reached 76.2% CH_4 and 20.4% CO_2 at 3 bar. When the pressure was further increased to 5 bar, the biogas content changed to 79.5% CH_4 and 15.3% CO_2, respectively (data not shown). In this study, at the end of phase 6 and 7 (11 bar), the CH_4 concentration in the headspace ranged from 87.4% to 88.5%, while the CO_2 content was between 11.5% and 12.6%. Hence, at the pressure of 11 bar, the CO_2 partial pressure was around 1.3 bar. Based on Henry's law, the theoretical CH_4 content should have been around 97% at 11 bar, which is higher than 88% measured in the high pressure reactor. However, the CH_4 content in the gas phase of the HPAD reactor is not solely dependent on Henry's law (water as a liquid) but also determined by other factors (i.e., ions in the liquid and CO_2 saturation). The gas composition in this study was similar to another study, where the CH_4 content was 75 ~ 86%, and CO_2 content was 14 ~ 25% at 20 bar (Lindeboom et al., 2016). To note, the pH in the reactors had the strongest impact on the gas composition. The decrease of pH will lead to a significantly lower absorption of CO_2 in the liquid phase, which could further influence the CH_4 content in the gas phase (Lemmer et al., 2015b).

The CH_4 content in the biogas from the NPAD reactor ranged from 55% to 64% during phase 2–5, which was higher than the stoichiometric yields. The difference might be explained because part of the CO_2 formed during the decomposition of organic matter (i.e., acetate or glucose) remains in solution as (bi)carbonate, resulting in a higher CH_4 content in the biogas of the NPAD reactor than the expected stoichiometric value (Nolla-ardèvol et al., 2015). In phase 6, the CH_4 content of the NPAD reactor decreased to 35%~42%, suggesting that the methanogens were at least partly inhibited under the applied conditions.

3.2. pH, alkalinity, and volatile fatty acids

The operational status of the AD process is reflected by pH, alkalinity (TA), and VFAs. The pH and TA of the HPAD reactor showed a rising trend (Fig. 2). The pH value of the HPAD reactor was lower than that of the NPAD reactor during phase 1–5, which was due to carbonic acid formation from a higher amount of dissolved CO_2 in the HPAD reactor. The pH of the samples was measured externally at atmospheric pressure and was not the real pH inside the HPAD reactor since part of the CO_2 escaped immediately when the samples were taken. Therefore, the pH inside the HPAD reactor was lower than the pH of the samples at atmospheric pressure. During phase 6 and 7, the pH of the NPAD reactor was lower than that of the HPAD reactor, which was ascribed to the high concentration of VFAs accumulated in the NPAD reactor (Fig. 2E). At the end of phase 4 and 5, only acetic acid could be detected in the NPAD reactor, which was 143 mg/L and 2285 mg/L in phase 4 and phase 5, respectively (Table 2). At the end of phase 6, the concentration of acetic acid, propionic acid, and butyric acid was 3212 mg/L, 178 mg/L, and 18 mg/L, respectively. The optimal pH range for methanogens is between 6.8 and 7.8. Thus, a pH of 8.2 ~ 8.4 caused by a high TA (phase 2–5 of the NPAD reactor) was too high for methanogens (Chen et al., 2015). The inhibited methanogens may fail to further convert acetic acid into biogas, resulting in the decline of pH in phase 6 and 7.

TA is related to pH in a complex manner and a suitable TA concentration is necessary to maintain optimal conditions in the AD process (Chen et al., 2015). And in this study, high TA was the main reason leading to the higher pH and VFAs concentration in the NPAD reactor. The substrates fed into the HPAD and NPAD reactors were the same in phase 2–5, but TA in the NPAD reactor was much higher than in the HPAD reactor (Fig. 2D). An optimal TA level ranges from 1.0 to 5.0 g/L. However, the TA of 10.0 g/L in the NPAD reactor was much higher than the upper limit of alkalinity (Osman and Sponza, 2005). Likewise, in a previous study, where Zhu et al. (2010) used 5% (w/w) NaOH to pretreat lignocellulosic biomass for subsequent AD. Although a better degradation of lignocellulose was achieved, high initial TA of the pretreated biomass (21.8 g/L) strongly inhibited the subsequent methanogenesis stage and led to VFAs accumulation (2000 mg/L acetic acid). Hence, choosing HPAD reactors instead of NPAD reactors to deal with wastewater containing high alkalinity could be an alternative. This idea is further illustrated below:

The TA of the samples is defined as its acid-neutralizing capacity (Lemmer et al., 2017; Lindeboom et al., 2012) and is expressed as:

$$\text{Total alkalinity} = 2[CO_3^{2-}] + [HCO_3^-] + [OH^-] + [VFA^-] - [H^+]$$

Under pressure, CO_2 dissolves into the liquid phase and is partly converted into carbonic acid. Henry's law describes the partitioning of CO_2 between the gas and liquid phases. In the calculations of TA, the K_H-value for hydration and dehydration reactions of CO_2 are important (log K_H = -1.6, all constants at 35 °C). H_2CO_3 first dissociates into HCO_3^-, when the pH value is above 8.5, it further dissociates into CO_3^{2-} (K_1 is first dissociation constant, log K_1 = −6.3; K_2 is second dissociation constant, log K_2 = −10.3) (Lemmer et al., 2015a; Lindeboom et al., 2012). The pH of the HPAD reactor is below 8, and therefore, the dissociation of HCO_3^- into CO_3^{2-} can be neglected (Lemmer et al., 2015a). The constant K_H is much higher than K_1 in HPAD, which means that the carbonic acid concentration is higher than the bicarbonate concentration in the HPAD reactor, and therefore the TA is lower than in the NPAD reactor.

3.3. Microbiology community structure

3.3.1. Comparison of community diversity

The alpha diversity measurements of Bacteria and Archaea are represented using the number of OTUs, Margalef index (*d*), Shannon diversity index (*H'*), and Simpson diversity index (1-*D*) (Table 3). An improved digester performance is correlated with high microbial richness (reflected by a high Margalef index) and evenness (reflected by a high Shannon or Simpson diversity index) (Tao et al., 2020;

Table 3
Alpha diversity metrics of the samples (The samples of the HPAD and NPAD reactors were taken at the end of phase 7.).

	Sample	OTUs	Margalef (*d*)	Shannon(*H'*)	Simpson(1-*D*)
Bacteria	Sludge	7719	32.84	3.69	0.93
	HPAD	4700	31.46	4.00	0.96
	NPAD	3636	27.44	3.97	0.95
Archaea	Sludge	109	2.56	1.94	0.77
	HPAD	497	4.19	1.71	0.68
	NPAD	1395	4.00	1.47	0.59

Venkiteshwaran et al., 2015). The community analysis was performed on samples taken from the inoculum sludge and at the end of phase 7 from the HPAD and NPAD reactors. The bacterial community in the HPAD reactor had slightly higher indices than those of the NPAD reactor. Samples from the NPAD reactor had lower indices for Archaea compared with the indices obtained from the HPAD reactor. This was consistent with its corresponding poor digester performance (i.e., low cumulative methane yield: 0.45 L, high TA: 6.5 g/L, and high VFAs: 7455, 451 and 52 mg/L for acetic acid, propionic acid and butyric acid, respectively, at the end of phase 7). In contrast, the HPAD reactor showed better performance, reflected by its higher indices for Margalef, Shannon and Simpson (4.19, 1.71, and 0.68, respectively).

3.3.2. Phylogenetic analysis of bacteria at the phylum level

Fourteen major phyla, each represented > 0.1% of the total bacterial sequences in at least one sample, were identified (Fig. 3A). Several phyla were found ubiquitous and predominant at the end of phase 7 and included Chloroflexi, Bacteroidetes, Proteobacteria, and Firmicutes. These phyla are widely present in AD reactors, and their existence is thought to be due to the role that they play in the degradation of complex polymeric organic compounds and fermentation of monomeric sugars in AD systems, and their ability to survive under high alkalinity conditions (Alirio et al., 2018; Sun et al., 2014; Venkiteshwaran et al., 2015).

Being the most predominant phylum, Chloroflexi accounted for 33.1% in the inoculated sludge. The relative abundance (RA) of Chloroflexi decreased slightly in both the HPAD and NPAD samples (24.7% and 24.3%, respectively). Chloroflexi and Proteobacteria (Alpha-, Beta-, Gamma-, and Delta-) are bacteria that can utilize glucose and VFAs (Ros et al., 2017). A lower RA of Proteobacteria in the HPAD reactor (16.9%) than in the NPAD reactor (20.3%) indicated that high pressure might influence the activity of this phylum. The members of the Delta-proteobacteria are dominant (12%) in the HPAD reactor, and they can syntrophically degrade propionate (i.e., *Syntrophus* and *Geobacter* species) (Ariesyady et al., 2007; Ito et al., 2012). In the NPAD reactor, Delta-proteobacteria accounted for only 1.2%, explaining the propionic acid accumulation in this reactor. The highest RA (18.9%) among the Proteobacteria in the NPAD reactor was due to the Gamma-proteobacteria. These bacteria usually show high hydrolytic activity, and they have a great adaptive capacity in an alkaline environment (Li et al., 2018; Tian et al., 2017).

The second-largest phylum, Bacteroidetes, accounted for 19.7% in the inoculated sludge. The RA of Bacteroidetes increased further to 26.1% and 28.8% in the samples from the HPAD and NPAD reactors, respectively. Bacteroidetes are dominant in mesophilic AD, and they can produce various lytic enzymes and acetic acid during the degradation of organic materials (Ros et al., 2017). Some members of Bacteroidetes are known for their survival in a high alkaline environment (soda lakes) (Lin et al., 2017; Nolla-ardèvol et al., 2015). Additionally, together with Proteobacteria and Chloroflexi, Bacteroidetes, are typical glucose degraders, which may explain their profusion in samples from the HPAD and NPAD reactors (Ito et al., 2012). Firmicutes had a much higher RA in the HPAD and in the NPAD reactors, reaching 24.5% and 21.9%, respectively. Some members of the Firmicutes are butyrate-oxidizing bacteria, while other Firmicutes species are capable of fermenting sugars, and are involved in homoacetogenesis and syntrophic oxidation of acetate (Guo et al., 2015; Nolla-ardèvol et al., 2015; Yi et al., 2014).

3.3.3. Phylogenetic analysis of bacteria at the genus level

There are more apparent differences between the HPAD and NPAD reactors at the bacterial genus level. The distribution patterns of 35 major bacterial genera in samples of the HPAD and NPAD reactors (each genus represented ≥ 0.5% of the total bacterial sequences) are presented in Fig. 3B. In the sample of the HPAD reactor, *Dehalogenimonas* (17.2%), *Cytophaga* (14.2%), *Syntrophus* (11.2%), *Acetivibrio* (7.4%), and *Clostridium* (5.9%) were found with a high RA. The presence of these five bacterial genera may suggest their robustness in a high pressure (11 bar) environment. The predominant genera in the NPAD reactor shared nothing in common with the genera obtained in the HPAD reactor except for *Cytophaga* (15.3%). The major bacteria genera in the NPAD reactor were *Pseudomonas* (16.5%), *Longilinea* (12%), *Bacillus* (7.8%), *Bellilinea* (6.5%), and *Proteiniphilum* (6.1%).

Based on their potential different metabolic pathways, the 35 major genera are clustered into three groups, VFAs producing (I), VFAs utilizing (II), and unknown (III).

Group I, VFAs producing group. Among all the known 127 bacterial strains in these samples, 64 strains can generate acetic acid, 35 strains can produce propionic acid, and 14 strains are butyric acid producers (Tao et al., 2020). Moreover, 8 kinds of archaeal genera can produce propionic acid through the succinate pathway. In the last phase, when glucose was used as a substrate, the NPAD reactor contained the highest RA of VFAs producing bacteria (68.76%) and had more acetic acid-producing and butyric acid-producing bacteria than the HPAD reactor (Fig. 3D and E). *Citrobacter* was only present in the HPAD reactor and *Proteiniphilum, Psychrobacter,* and *Bacillus*, on the other hand, were only found in the NPAD reactor. *Longilina* and *Bacillus* are both carbohydrate-utilizing bacteria that were present in a high RA in the NPAD reactor (Alirio et al., 2018). *Pseudomonas* may use the valine degradation pathway to generate butyric acid via isobutyryl-CoA during glucose fermentation and could survive under high alkalinity conditions (Wainaina et al., 2019). That may explain why *Pseudomonas* dominated in the NPAD reactor. *Cytophaga* was present in a high RA in both the HPAD and NPAD reactors; they are common cellulolytic bacteria with some species capable of rapidly degrading cellobiose or glucose and producing VFAs (Tao et al., 2020). The RA of the genera *Gracilibacter* was higher in the HPAD reactor (5.53%) than in the NPAD reactor. In a previous study, it was reported that *Gracilibacter* was only functional in the acidogenesis stage and might produce short-chain fatty acids and H_2 (Usman et al., 2019).

Group II, VFAs utilizing group. Syntrophic bacteria are VFAs utilizing bacteria and were present in a higher RA in the HPAD reactor (14.62%) than in the NPAD reactor (3.00%) (Fig. 3D). Probably, the enrichment of syntrophic bacteria in the HPAD reactor could partly answer the question why there were almost no VFAs detected in the HPAD reactor throughout phase 1–7. Although syntrophic bacteria account for a relatively small percentage in AD, their presence is critical and they can perform the rate-limiting step to maintain a rapid and stable AD process (Hao et al., 2020; Venkiteshwaran et al., 2015). Thus, the syntrophic relationships between bacteria and methanogens are necessary for a stable AD (Hao et al., 2020; Tao et al., 2020; Usman et al., 2019). In our study, *Syntrophus* was found to be the dominant syntrophic bacterium in the HPAD reactor (11.19%), and could syntrophically work with H_2, formate and/or acetate utilizing methanogens to degrade butyrate, or other VFAs (Grabowski et al., 2005). The lack of *Syntrophus* in the NPAD reactor (0.91%) may partly lead to the accumulation of VFAs. Previously, Grabowski et al. (2005) used fluorescent in situ hybridization to demonstrate the formation of a close spatial association between *Syntrophus* and the methanogenic archaea (*Methanocalculus* and *Methanosaeta*). The high RA of *Syntrophus*

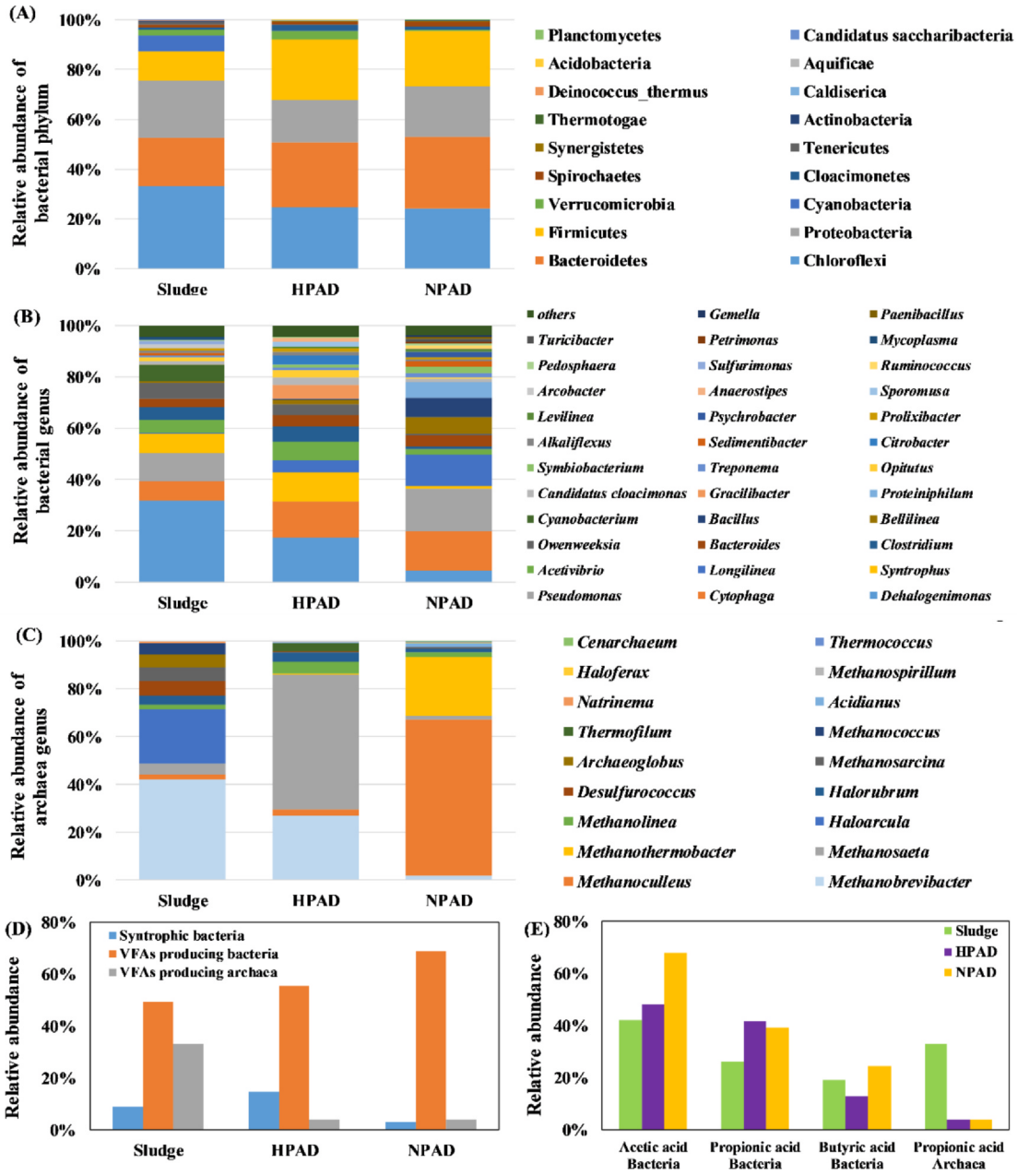


Fig. 3. (A) Relative abundance of bacteria at the phylum level. (B) Relative abundance of bacteria at the genus level ≥ 0.5%. (C) Relative abundance of archaea at the genus level. (D) Relative abundance of syntrophic bacteria and VFAs-producing bacteria and archaea. (E) Relative abundance of different acid-producing bacteria/archaea in the inoculum sludge, the HPAD and NPAD reactors. Notes:The syntrophic bacteria were *Syntrophus, Candidatus Cloacimonas, Geobacter, Syntrophorhabdus, Pelotomaculum, Smithella* and *Aminobacterium*. The genera of VFAs-producing bacteria were classified according to the short-chain fatty acids database (http://fungene.cme.msu.edu/scfa), with at least one strain of a particular genus being able to produce acetic acid, propionic acid, or butyric acid.

(11.19%) and *Methanosaeta* (56.3%) in the HPAD reactor indicated a higher degradation rate of intermediate products (VFAs). The genus *Clostridium* could contribute to the hydrogen production because various well-defined syntrophic hydrogen-producing bacteria have been affiliated with this genus; they could syntrophically work with hydrogenotrophic methanogens (HM) (Lin et al., 2017; Ros et al., 2017). The correlation between a high RA of *Clostridium* and an efficient and stable AD performance was reported previously (Ros et al., 2017). In this study, it was shown that *Clostridium* was found more abundant in the HPAD reactor (5.9%) than in the NPAD reactor (1.2%). *Candidatus Cloacimonas (Candidatus Cloacimonas acidaminovorans)* is a hydrogen-producing syntrophic bacterium (Guo et al., 2015). Similarly, a slightly higher RA of *Candidatus Cloacimonas* was found in the HPAD reactor (2.8%), than in the NPAD reactor (1.4%). Hence, compared with the normal pressure conditions, high pressure favors the growth of syntrophic bacteria. *Sporomusa* is a homoacetogenic bacterium and was only detected in the HPAD reactor, accounting for 1.9%. Homoacetogens are H_2 consumers that might receive hydrogen produced by bacteria from Group I. If methanogens are inhibited, then the accumulation of H_2 might result in the consumption of H_2 by the homoacetogens (Wahid et al., 2019). The high pressure resulted in an increase of P_{H2} in the HPAD reactor, which might explain the presence of *Sporomusa* in the HPAD reactor. This result was comparable to a previous study showing that the addition of H_2 in an AD reactor resulted in the enrichment of homoacetogens (Wahid et al., 2019). Besides, the higher concentration of H_2 in the HPAD reactor could not only be used by HM to produce CH_4, but also by the acetoclastic methanogens (AM) to degrade acetate that was produced by the homoacetogens.

Group III, function unknown group. *Dehalogenimonas* from the phylum Chloroflexi was identified to be a genus of organohalide-respiring bacteria, which could couple dehalogenation to a respiration process required for growth (Molenda et al., 2016). *Dehalogenimonas* had the highest RA in the sludge sample (31.7%) but was not the highest abundant bacterium in the samples of the HPAD and NPAD reactors.

To summarize, the distribution patterns of Group I and II genera were most likely the result of VFAs production and VFAs degradation. In particular, the enriched bacteria in the HPAD reactor were categorized in Group II, and they could increase the conversion of VFAs into acetate, H_2 and CO_2. Whereas the NPAD reactor contained more bacteria from Group I, which could accelerate the degradation of glucose to VFAs. Due to the lack of a sufficient number of bacteria from Group II, the NPAD reactor was vulnerable to VFAs accumulation.

3.3.4. Phylogenetic analysis of Archaea at the genus level

A total of 18 genera were identified in the inoculum sludge and in the two reactors. Five Archaea, i.e., *Methanobrevibacter, Methanosaeta, Methanoculleus, Methanothermobacter,* and *Haloarcula* were dominant (Fig. 3C), together accounting for 71.56 ~ 93.48% of the total Archaea. However, the distribution pattern was different in the three samples and was consistent with the alpha diversity of Archaea. *Methanobrevibacter* (26.8%) and *Methanosaeta* (56.3%) prevailed in the HPAD reactor while *Methanoculleus* (65.3%) and *Methanothermobacter* (24.3%) were dominant in the NPAD reactor.

Methanosaeta is one of the few Archaea that can metabolize acetic acid to produce methane in mesophilic AD, and also dominated in previous HPAD studies (Li et al., 2017; Lindeboom et al., 2016). The substrate during phase 2–6 was acetate in the HPAD reactor, which laid the foundation for the increase of *Methanosaeta* in the reactor. *Methanosaeta* has a competitive advantage over other acetate-metabolizing microorganisms due to its high affinity for acetate (Li et al., 2020; Ros et al., 2017; Venkiteshwaran et al., 2015). Moreover, the filamentous morphology of *Methanosaeta* may be of importance for the formation of the microbial floc structure that helps to stabilize the HPAD systems further (Venkiteshwaran et al., 2015). *Methanosaeta concilii* was abundantly present (50.9%) in the HPAD reactor. This was in line with Lindeboom et al. (2016), who found that this species was abundantly present in an HPAD fed with glucose. *M. concilii* has been detected in high-pressure subsurface gas, oil reservoirs, and aquatic sediments (Borrel et al., 2012; Grabowski et al., 2005; Lindeboom et al., 2016), indicating that *M. concilii* is a pressure-tolerant methanogen. Presumably, *Methanosaeta* works with *Syntrophus* and homoacetogens, i.e., *Sporomusa,* in the HPAD reactor to convert acetate into biogas, as explained before (bacterial Section 3.3.3). *Methanosarcina* is an important Archaea that can generate CH_4 through different metabolic pathways. But it did not prevail among these three samples, especially in the sample from the HPAD reactor. Li et al. (2017) also found that the RA of *Methanosarcina* decreased at elevated pressure. It is likely that *Methanosarcina* was not able to compete with *Methanosaeta* for acetate in the HPAD reactor, and also may be inhibited by the high alkalinity/VFAs in the NPAD reactor, which was in agreement with previous studies (Sun et al., 2014; Wahid et al., 2019). The lack of *Methanosaeta* and *Methanosarcina* strongly reduced the efficiency of acetic acid consumption (Lv et al., 2020), and resulted in VFAs accumulation in the NPAD reactor.

The second-largest genus, *Methanobrevibacter* has a share of 26.8% among the Archaea in the sample of the HPAD reactor, but its RA was only 1.9% in the sample of the NPAD reactor. This genus can use H_2/CO_2 or formate to produce CH_4, suggesting that *Methanobrevibacter* is one of the few HM that can survive under high pressure. This methanogen can also survive in acidic conditions. Some species, such as *Methanobrevibacter acididurans* were found in a reactor treating slurry from an acidogenic digester at pH 5.0 (Savant et al., 2002). It complied well with the situation in our case since the pH of the HPAD reactor was lower than the pH in the NPAD reactor because of the formation of carbonic acid in the liquid phase. Hence, these HM (*Methanobrevibacter, Methanolinea, Methanoculleus, Methanospirillum,* and *Methanothermobacter*) and their role in the compensation metabolism for AM in the HPAD reactor cannot be overlooked, especially *Methanobrevibacter* (Li et al., 2020; Zhao et al., 2018). These HM converted CO_2 and H_2 into CH_4, which decreased the P_{H2} to maintain a stable HPAD reactor.

A high RA of *Methanoculleus* and *Methanothermobacter* in the NPAD reactor was observed in this study. These genera are HM and could survive in conditions where VFAs accumulate (Hori et al., 2006; Lin et al., 2017; Zhao et al., 2018), and *Methanoculleus* was also found in a high alkalinity AD process (Sun et al., 2014). The high VFAs, caused by high alkalinity, was most likely the main factor in changing the methanogenic communities in the NPAD reactor. It has been reported before that *Methanothermobacter* dominated in a reactor that was operated at thermophilic conditions (Lin et al., 2017). However, in this mesophilic AD experiment, *Methanothermobacter thermautotrophicus* was also detected in the NPAD reactor, accounting for 24.3% of the total Archaea. In the HPAD reactor, *Methanosarcina* and *Methanococcus* were not detected, although they were present in the inoculating sludge and in the NPAD reactor. This suggests that these two HM cannot tolerate high pressure, but this hypothesis needs further investigation.

Compared with the HPAD, there were more HM (93.4%) and less AM (1.7%) in the NPAD reactor. To date, HM tend to be more abundant in reactors operating at special conditions (i.e. low pH, high VFAs concentration, highly alkaline environments) (Nolla-ardèvol et al., 2015; Town et al., 2014; Zhao et al., 2018). Besides, in the NPAD reactor, there were more acetic acid-producing bacteria (67.8%), and almost no AM (1.7%) present; thus, the balance between these acetic acid-producing and acetic acid-consuming microorganisms was broken, which further led to acetic acid accumulation in this reactor. Previous reports also illustrated that AD reactors were stable and healthy when AM were present in a high RA (Li et al., 2020; Town et al., 2014; Usman et al., 2019). Moreover, the co-existence of both acetoclastic and hydrogenotrophic methanogens could provide a more robust microbial community (Li et al., 2020; Town et al., 2014; Usman et al., 2019).

To summarize, both acetoclastic and hydrogenotrophic methanogens were abundantly present in the HPAD reactor. The NPAD reactor was vulnerable to VFAs accumulation, most likely due to the low abundance of AM.

4. Conclusions

In the HPAD reactor, the CH_4 content was 88.5% when the pressure reached up to 11 bar. Compared with the NPAD reactor, the HPAD reactor was better able to deal with the high alkalinity acetate-containing synthetic wastewater. The advantage of HPAD was reflected by stable operation indexes and functional microbial guilds such as *Syntrophus, Methanosaeta* (*M. concilii*) and *Methanobrevibacter*. The HPAD reactor can be used in the future to treat alkaline industrial wastewater and upgrade biogas to green gas in a single step.

CRediT authorship contribution statement

Jing Zhao: Conceptualization, Methodology, Investigation, Writing - original draft. **Yu Li:** Methodology, Visualization, Writing - original draft. **Clara Marandola:** Investigation. **Janneke Krooneman:** Supervision, Writing - review & editing. **Gert Jan Willem Euverink:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

J. Zhao and Y. Li were financed by the China Scholarship Council (CSC) under the grant numbers 201706350276 (Zhao) and 201600090213 (Li). The other authors did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.124101.

References

Alirio, W., Liu, H., Wu, M., 2018. Roles of acid-producing bacteria in anaerobic digestion of waste activated sludge. Front. Environ. Sci. Eng. 12, 158–168.
Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H., Kougias, P.G., 2018. Biogas upgrading and utilization: Current status and perspectives. Biotechnol. Adv. 36, 452–466.
APHA, Standard, 2005. Methods for the examination of water and wastewater, twentiethed. American Public Health Association (APHA, AWWA, and WPCF), Washington DC.
Ariesyady, H.D., Ito, T., Okabe, S., 2007. Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester. Water Res. 41, 1554–1568.
Borrel, G., Colombet, J., Prangishvili, D., 2012. Unexpected and novel putative viruses in the sediments of a deep-dark permanently anoxic freshwater habitat. ISME J. 6, 2119–2127.
Budzianowski, W.M., Postawa, K., 2017. Renewable energy from biogas with reduced carbon dioxide footprint: Implications of applying different plant configurations and operating pressures. Renew. Sustain. Energy Rev. 68, 852–868.
Chen, S., Zhang, J., Wang, X., 2015. Effects of alkalinity sources on the stability of anaerobic digestion from food waste. Waste Manag. Res. 33, 1033–1040.
Chen, Y., Rößler, B., Zielonka, S., Lemmer, A., Wonneberger, A., Jungbluth, T., 2014a. The pressure effects on two-phase anaerobic digestion. Appl. Energy. 116, 409–415.
Chen, Y., Rößler, B., Zielonka, S., Wonneberger, A., Lemmer, A., 2014b. Effects of organic loading rate on the performance of a pressurized anaerobic filter in two-phase anaerobic digestion. Energies. 7, 736–750.
Savant, D.V., Shouche, Y.S., Prakash, S., Ranade, D.R., 2002. Methanobrevibacter acididurans sp.nov., a novel methanogen from a sour anaerobic digester. Int. J. Syst. Evol. Microbiol. 52, 1081–1087.
Filer, J., Ding, H.H., Chang, S., 2019. Biochemical methane potential (BMP) assay method for anaerobic digestion research. Water. 11, 1–29.
Grabowski, A., Blanchet, D., Christian, J., 2005. Characterization of long-chain fatty-acid-degrading syntrophic associations from a biodegraded oil reservoir. Res. Microbiol. 156, 814–821.
Guo, J., Peng, Y., Ni, B., Han, X., Fan, L., Yuan, Z., 2015. Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing. Microb. Cell Fact. 14, 1–11.
Hao, L., Yssing, T., Caitlin, M., Singleton, M., Dottorini, G., Hansen, R., Mads, K., Per, A., Nielsen, H., Simonsen, M., 2020. Novel syntrophic bacteria in full-scale anaerobic digesters revealed by genome-centric metatranscriptomics. ISME J. 14, 906–918.
Hori, T., Haruta, S., Ueno, Y., Ishii, M., Igarashi, Y., 2006. Dynamic transition of a methanogenic population in response to the concentration of volatile fatty acids in a thermophilic anaerobic digester. Appl. Environ. Microbiol. 72, 1623–1630.
Ito, T., Yoshiguchi, K., Dwi, H., Okabe, S., 2012. Identification and quantification of key microbial trophic groups of methanogenic glucose degradation in an anaerobic digester sludge. Bioresour. Technol. 123, 599–607.
Latif, M.A., Mehta, C.M., Batstone, D.J., 2018. Enhancing soluble phosphate concentration in sludge liquor by pressurised anaerobic digestion. Water Res. 145, 660–666.
Lemmer, A., Chen, Y., Lindner, J., Wonneberger, A.M., Zielonka, S., Oechsner, H., Jungbluth, T., 2015a. Influence of different substrates on the performance of a two-stage high pressure anaerobic digestion system. Bioresour. Technol. 178, 313–318.
Lemmer, A., Chen, Y., Wonneberger, A., 2015b. Integration of a water scrubbing technique and two-stage pressurized anaerobic digestion in one process. Energies. 8, 2048–2065.
Lemmer, A., Merkle, W., Baer, K., Graf, F., 2017. Effects of high-pressure anaerobic digestion up to 30 bar on pH-value, production kinetics and specific methane yield. Energy. 138, 659–667.
Li, X., Liu, G., Liu, S., Ma, K., Meng, L., 2018. The relationship between volatile fatty acids accumulation and microbial community succession triggered by excess sludge alkaline fermentation. J. Environ. Manage. 223, 85–91.
Li, Y., Achinas, S., Zhao, J., Geurkink, B., Krooneman, J., Euverink, G.J.W., 2020. Co-digestion of cow and sheep manure: Performance evaluation and relative microbial activity. Renew. Energy. 153, 553–563.
Li, Y., Liu, H., Yan, F., Su, D., Wang, Y., Zhou, H., 2017. High-calorific biogas production from anaerobic digestion of food waste using a two-phase pressurized biofilm (TPPB) system. Bioresour. Technol. 224, 56–62.
Lin, L., Yu, Z., Li, Y., 2017. Sequential batch thermophilic solid-state anaerobic digestion of lignocellulosic biomass via recirculating digestate as inoculum – Part II: Microbial diversity and succession. Bioresour. Technol. 241, 1027–1035.
Lindeboom, R.E.F., Fermoso, F.G., Weijma, J., Zagt, K., Lier, J.B.V., 2011. Autogenerative high pressure digestion: anaerobic digestion and biogas upgrading in a single step reactor system. Water Sci. Technol. 64, 647–653.
Lindeboom, R.E.F., Ferrer, I., Weijma, J., Lier, J.B.V., 2013a. Silicate minerals for CO_2 scavenging from biogas in autogenerative high pressure digestion. Water Res. 47, 3742–3751.
Lindeboom, R.E.F., Ferrer, I., Weijma, J., Lier, J.B.V., 2013b. Effect of substrate and cation requirement on anaerobic volatile fatty acid conversion rates at elevated biogas pressure. Bioresour. Technol. 150, 60–66.
Lindeboom, R.E.F., Shin, S.G., Weijma, J., 2016. Piezo-tolerant natural gas-producing microbes under accumulating pCO_2. Biotechnol. Biofuels. 9, 1–19.
Lindeboom, R.E.F., Weijma, J., Lier, J.B.V., 2012. High-calorific biogas production by selective CO_2 retention at autogenerated biogas pressures up to 20 bar. Environ. Sci. Technol. 46, 1895–1902.
Lv, N., Zhao, L., Wang, R., Ning, J., Pan, X., Li, C., Cai, G., 2020. Novel strategy for relieving acid accumulation by enriching syntrophic associations of syntrophic fatty acid-oxidation bacteria and H_2/formate-scavenging methanogens in anaerobic digestion. Bioresour. Technol. 313, 123702.
Merkle, W., Baer, K., Haag, N.L., Zielonka, S., Ortloff, F., Graf, F., Lemmer, A., 2017a. High-pressure anaerobic digestion up to 100 bar: influence of initial pressure on production kinetics and specific methane yields. Environ. Technol. 38, 337–344.
Merkle, W., Baer, K., Lindner, J., Zielonka, S., Ortloff, F., Graf, F., Kolb, T., Jungbluth, T., Lemmer, A., 2017b. Influence of pressures up to 50 bar on two-stage anaerobic digestion. Bioresour. Technol. 232, 72–78.
Molenda, O., Quaile, A.T., Edwards, E.A., 2016. Dehalogenimonas sp. strain WBC-2 genome and identification of its trans-dichloroethene reductive dehalogenase. TdrA. Appl. Environ. Microbiol. 82, 40–50.
Nolla-ardèvol, V., Strous, M., Tegetmeyer, H.E., 2015. Anaerobic digestion of the microalga Spirulina at extreme alkaline conditions: biogas production, metagenome, and metatranscriptome. Front. Microbiol. 6, 1–21.
Omar, B., Abou-shanab, R., El-gammal, M., Fotidis, I.A., Kougias, P.G., Zhang, Y., Angelidaki, I., 2018. Simultaneous biogas upgrading and biochemicals production using anaerobic bacterial mixed cultures. Water Res. 142, 86–95.
Osman, N., Sponza, D.T., 2005. Effect of alkalinity on the performance of a simulated landfill bioreactor digesting organic solid wastes. Chemosphere. 59, 871–879.
Rao, A.G., Reddy, T.S.K., Prakash, S.S., Vanajakshi, J., Joseph, J., Sarma, P.N., 2007. pH regulation of alkaline wastewater with carbon dioxide: a case study of treatment of brewery wastewater in UASB reactor coupled with absorber. Bioresour. Technol. 98, 2131–2136.
Atlas, R.M., 2004. Handbook of microbiological media. third edition.
Ros, M., Souza, J.D., Filho, O., Dolores, M., Murcia, P., Angeles, M., Moral, R., Dolores, M., Belen, A., Santisima-trinidad, L., Antonio, J., 2017. Mesophilic anaerobic digestion of pig slurry and fruit and vegetable waste : Dissection of the microbial community structure. J. Clean. Prod. 156, 757–765.
Sun, R., Xing, D., Jia, J., Zhou, A., Zhang, L., Ren, N., 2014. Methane production and microbial community structure for alkaline pretreated waste activated sludge. Bioresour. Technol. 169, 496–501.
Tao, Y., Evren, M., Ghasimi, D.S.M., Ozgun, H., Wang, H., Zhang, X., Guo, M., Yang, Y., Stuckey, D.C., Lier, J.B.V., 2020. Biogas productivity of anaerobic digestion process is governed by a core bacterial microbiota. Chem. Eng. J. 380, 1–10.
Tian, H., Liu, J., Feng, T., Li, H., Wu, X., Li, B., 2017. Assessing the performance and microbial structure of biofilms adhering on aerated membranes for domestic saline sewage treatment. RSC Adv. 7, 27198–27205.
Town, J.R., Links, M.G., Fonstad, T.A., Dumonceaux, T.J., 2014. Molecular characterization of anaerobic digester microbial communities identifies microorganisms that correlate to reactor performance. Bioresour. Technol. 151, 249–257.
Usman, M., Hao, S., Chen, H., Ren, S., Tsang, D.C.W., 2019. Molecular and microbial insights towards understanding the anaerobic digestion of the wastewater from hydrothermal liquefaction of sewage sludge facilitated by granular activated carbon (GAC). Environ. Int. 133, 1–12.
Venkiteshwaran, K., Bocher, B., Maki, J., Zitomer, D., 2015. Relating anaerobic digestion microbial community and process function. Microbiol. insights. 8, 37–44.
Wahid, R., Mulat, D.G., Gaby, J.C., Horn, S.J., 2019. Effects of H_2:CO_2 ratio and H_2 supply fluctuation on methane content and microbial community composition during in-situ biological biogas upgrading. Biotechnol. Biofuels. 12, 1–15.
Wainaina, S., Awasthi, M.K., Mohammad, J., 2019. Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review. Bioengineered. 10, 437–458.
Yi, J., Dong, B., Jin, J., Dai, X., 2014. Effect of increasing total solids contents on anaerobic digestion of food waste under mesophilic conditions: performance and microbial characteristics analysis. PLOS One. 9, 1–10.
Zhao, J., Westerholm, M., Qiao, W., Yin, D., Bi, S., Jiang, M., Dong, R., 2018. Impact of temperature and substrate concentration on degradation rates of acetate, propionate and hydrogen and their links to microbial community structure. Bioresour. Technol. 256, 44–52.
Zhu, J., Wan, C., Li, Y., 2010. Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. Bioresour. Technol. 101, 7523–7528.